FORM PTO-1390 (REV. 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: GJE-71

U S APPLICATION NO. (If known, see 37 CFR 1 5): 09/868195

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/04376	22 Dec 1999	22 Dec 1998 (see no. 20 below)

TITLE OF INVENTION: Outer SurfaceProteins, Their Genes, And Their Use

APPLICANT(S) FOR DO/EO/US: Martin John Glenton Hughes, Joseph David Santangelo, Jonathan Douglas Lane, Robert Feldman, Joanne Christine Moore, Richard James Dobson, Paul Everest, Joanne Henwood, Gordon Dougan, Rebecca Kerry Wilson

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☑ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☑ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b ☑ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☑ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)), unsigned.
10. ☐ An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☑ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 37 CFR 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☑ Other items or information:

Priority dates: 22 December 1998; 20 January 1999; 12 April 1999; 24 May 1999; and 23 September 1999.

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/868195	PCT/GB99/04376	GJE-71

21. ☑ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**

	CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$0.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$
Total claims	19 - 20 =		x **$18.00**	$0.00
Independent claims	9 - 3 =	6	x **$80.00**	$480.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$0.00
TOTAL OF ABOVE CALCULATIONS =				$1.340.00
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$0.00
SUBTOTAL =				$1.340.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$0.00
TOTAL NATIONAL FEE =				$1.340.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$0.00
TOTAL FEES ENCLOSED =				$1.340.00
			Amount to be refunded:	$
			charged:	$

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☑ Please charge my Deposit Account No. 19-0065 in the amount of $ 1,340.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☑ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0065 . A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

CORRESPONDENCE ADDRESS:

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David Saliwanchik
SIGNATURE

June 15, 2001
DATE

David R. Saliwanchik
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31,794
REGISTRATION NUMBER

PRELIMINARY AMENDMENT
Patent Application

June 15, 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Martin John Glenton Hughes, Joseph David Santangelo, Jonathan Douglas Lane, Robert Feldman, Joanne Christine Moore, Richard James Dobson, Paul Everest, Caroline Joanne Henwood, Gordon Dougan, Rebecca Kerry Wilson

Docket No. : GJE-71

For : Outer Surface Proteins, Their Genes, And Their Use

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please amend the above-identified patent application as follows:

In the Specification

After page 11: Please insert as new page 12 the attached Abstract of the Disclosure.

In the Claims

The following amendments are made with respect to the claims in international application PCT/GB99/04376. Please cancel claims 1-12 and add the following claims to read as follows:

13. A peptide encoded by a polynucleotide sequence wherein said polynucleotide sequence comprises a gene, obtainable from a Group B *Streptococcus*, selected from the group consisting of MS4, MS10, MS11, MS14 and MS16; or said polynucleotide sequence comprises a homologue or a functional fragment of one of said Group B *Streptococcus* genes.

14.The peptide, according to claim 13, comprising an amino acid sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10 and 12.

15. A polynucleotide wherein said polynucleotide sequence comprises a gene, obtainable from a Group B *Streptococcus*, selected from the group consisting of MS4, MS10, MS11, MS14 and MS16; or said polynucleotide sequence comprises a homologue or a functional fragment of one said Group B *Streptococcus* genes.

16. A polynucleotide which encodes a peptide selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10 and 12.

17. A host transformed to express a peptide encoded by a polynucleotide sequence wherein said polynucleotide sequence comprises a gene, obtainable from a Group B *Streptococcus*, selected from the group consisting of MS4, MS10, MS11, MS14 and MS16; or said polynucleotide sequence comprises a homologue or a functional fragment of one of said Group B *Streptococcus* genes.

18. The host, according to claim 17, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10 and 12.

19. A vaccine comprising either 1) a peptide encoded by a polynucleotide sequence wherein said polynucleotide sequence comprises a gene, obtainable from a Group B *Streptococcus*, selected from the group consisting of MS4, MS10, MS11, MS14 and MS16; or said polynucleotide sequence comprises a homologue or a functional fragment of one of said Group B *Streptococcus* genes; or 2) a means for expressing said peptide.

20. The method, according to claim 19, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10 and 12.

21. A method for screening for potential drugs, wherein said method comprises the use of a peptide encoded by a polynucleotide sequence wherein said polynucleotide sequence comprises a gene, obtainable from a Group B *Streptococcus*, selected from the group consisting of MS4, MS10, MS11, MS14 and MS16; or said polynucleotide sequence comprises a homologue or a functional fragment of one of said Group B *Streptococcus* genes.

22. The method, according to claim 21, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10 and 12.

23. A method for the detection of virulence, wherein said method comprises the use of a peptide encoded by a polynucleotide sequence wherein said polynucleotide sequence comprises a gene, obtainable from a Group B *Streptococcus*, selected from the group consisting of MS4, MS10, MS11, MS14 and MS16; or said polynucleotide sequence comprises a homologue or a functional fragment of one of said Group B *Streptococcus* genes.

24. The method, according to claim 22, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10 and 12.

25. A method for the treatment or prevention of a condition associated with bacterial infection, wherein said method comprises administering to a patient in need of such treatment or prevention, an effective amount of a peptide encoded by a polynucleotide sequence wherein said polynucleotide sequence comprises a gene, obtainable from a Group

B *Streptococcus*, selected from the group consisting of MS4, MS10, MS11, MS14 and MS16; or said polynucleotide sequence comprises a homologue or a functional fragment of one of said Group B *Streptococcus* genes.

26. The method, according to claim 25, wherein the infection is a Group B *Streptococcal* infection.

27. The method, according to claim 25, wherein the infection is a local infection.

28. The method, according to claim 25, wherein the infection is a urinary tract infection.

29. The method, according to claim 25, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10 and 12.

30. An antibody raised against a peptide encoded by a polynucleotide sequence wherein said polynucleotide sequence comprises a gene, obtainable from a Group B *Streptococcus*, selected from the group consisting of MS4, MS10, MS11, MS14 and MS16; or said polynucleotide sequence comprises a homologue or a functional fragment of one of said Group B *Streptococcus* genes.

31. The antibody, according to claim 30, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10 and 12.

Remarks

Claims 1-12 have been canceled and new claims 13-31 have been added.

No new matter has been added by these amendments.

The Commissioner is hereby authorized to charge any fees under 37 CFR 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Respectfully submitted,

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DRS/la
Attachment: Abstract of the Disclosure

OUTER SURFACE PROTEINS, THEIR GENES, AND THEIR USE

Field of the Invention

This invention relates to the identification of outer surface proteins, their genes, and their use. More particularly, it relates to their use in therapy, for immunisation and in screening for drugs.

Background to the Invention

Group B *Streptococcus* (GBS), also known as *Streptococcus agalactiae*, is the causative agent of various conditions. In particular, GBS causes:

Early onset neonatal infection.

This infection usually begins *in utero* and causes severe septicaemia and pneumonia in infants, which is lethal if untreated and even with treatment is associated with a 10-20% mortality rate.

Late onset neonatal infection.

This infection occurs in the period shortly after birth until about 3 months of age. It causes a septicaemia, which is complicated by meningitis in 90% of cases. Other focal infections also occur including osteomyelitis, septic arthritis, abscessés and endopthalmitis.

Adult infections.

These appear to be increasingly common and occur most frequently in women who have just delivered a baby, the elderly and the immunocompromised. They are characterised by septicaemia and focal infections including osteomyelitis, septic arthritis, abscesses and endopthalmitis.

Urinary tract infections.

GBS is a cause of urinary tract infections and in pregnancy accounts for about 10% of all infections.

Veterinary infections.

GBS causes chronic mastitis in cows. This, in turn, leads to reduced milk production and is therefore of considerable economic importance.

GBS infections can be treated with antibiotics. However, immunisation is preferable. It is therefore desirable to develop an immunogen that could be used in a therapeutically-effective vaccine.

Summary of the Invention

The present invention is based on the identification of a series of genes in GBS, and also related organisms, the products of which may be associated with the outer surface of the organism and may therefore be useful as targets for immunotherapy.

According to one aspect of the invention, a peptide is encoded by an operon including any of the genes identified herein as MS4, MS10, MS11, MS14 and MS16, obtainable from Group B *Streptococcus*, or a homologue or functional fragment thereof. Such a peptide is suitable for therapeutic use, e.g. when isolated.

The term "functional fragments" is used herein to define a part of the gene or peptide which retains the activity of the whole gene or peptide. For example, a functional fragment of the peptide may be used as an antigenic determinant, useful in a vaccine or in the production of antibodies.

A gene fragment may be used to encode the active peptide. Alternatively, the gene fragment may have utility in gene therapy, targetting the wild-type gene *in vivo* to exert a therapeutic effect.

A peptide according to the present invention may comprise any of the amino acid sequences identified herein as SEQ ID NOS. 2, 4, 6, 8, 10 and 12, or a functional fragment thereof.

Because of the extracellular or cell surface location, the peptides of the present invention may be suitable candidates for the production of therapeutically-effective vaccines against GBS. The term "therapeutically-effective" is intended to include the prophylactic effect of vaccines. For example, a vaccine may comprise a peptide according to

the invention, or the means for its expression, for the treatment of infection.

This vaccine may be administered to females either prior to or during pregnancy to protect mother and neonate against infection by GBS.

According to another aspect of the invention, the peptides or genes may be used for screening potential antimicrobial drugs or for the detection of virulence.

A further aspect of this invention is the use of any of the products identified herein, for the treatment or prevention of a condition associated with infection by a Group B *Streptococcal* strain.

Although the protein has been described for use in the treatment of patients, veterinary uses of the products of the invention are also considered to be within the scope of the present invention. In particular, the peptides or the vaccines may be used in the treatment of chronic mastitis, especially in cows.

Description of the Invention

The present invention is described with reference to Group B *Streptococcal* strain M732. However, all the GBS strains and many other bacterial strains are likely to include related peptides or proteins having amino acid sequence homology with the peptide of M732. Organisms likely to contain the peptides include, but are not limited to, *S. pneumoniae*, *S. pyogenes*, *S. suis*, *S. milleri*, Group C and Group G *Streptococci* and *Enterococci*. Vaccines to each of these may be developed in the same way as described for GBS.

Preferably, the peptides that may be useful for the production of vaccines have greater than 40% sequence similarity with the peptides identified herein. More preferably, the peptides have greater than 60% sequence similarity. Most preferably, the peptides have greater than 80% sequence similarity, e.g. 95% similarity.

Having characterised a gene according to the invention, it is possible to use the gene sequence to

establish homologies in other microorganisms. In this way it is possible to determine whether other microorganisms have similar outer surface products. Sequence homologies may be established by searching in existing databases, e.g. EMBL or Genbank.

Peptides or proteins according to the invention may be purified and isolated by methods known in the art. In particular, having identified the gene sequence, it will be possible to use recombinant techniques to express the genes in a suitable host. Active fragments and homologues can be identified and may be useful in therapy. For example, the peptides or their active fragments may be used as antigenic determinants in a vaccine, to elicit an immune response. They may also be used in the preparation of antibodies, for passive immunisation, or diagnostic applications. Suitable antibodies include monoclonal antibodies, or fragments thereof, including single chain fv fragments. Methods for the preparation of antibodies will be apparent to those skilled in the art.

The preparation of vaccines based on attenuated microorganisms is known to those skilled in the art. Vaccine compositions can be formulated with suitable carriers or adjuvants, e.g. alum, as necessary or desired, and used in therapy, to provide effective immunisation against Group B *Streptococci* or other related microorganisms. The preparation of vaccine formulations will be apparent to the skilled person.

More generally, and as is well known to those skilled in the art, a suitable amount of an active component of the invention can be selected, for therapeutic use, as can suitable carriers or excipients, and routes of administration. These factors will be chosen or determined according to known criteria such as the nature/severity of the condition to be treated, the type or health of the subject etc.

The products of the present invention were identified as follows:

Todd-Hewitt broth was inoculated with GBS and allowed to grow overnight at 37°C. The cells were harvested by centrifugation and washed with Phosphate Buffered Saline (PBS). The cells were resuspended in an osmotic buffer (20%(w/v) Sucrose, 20mM Tris-HCl pH 7.0, 10mM $MgCl_2$) containing protease inhibitors (1 mM PMSF, 10 μM Iodoeacetic Acid, 10 mM 1,10-Phenanthroline, 1 μM Pepstatin A) and Mutanolysin at a final concentration of 4 Units per microlitre. This was incubated (shaking) at 37°C for 2 hours.

Cells and debris were removed first by high speed centrifugation, then ultra-centrifugation for 1 hour. The resultant supernatant containing cell wall proteins was concentrated under pressure using an ultrafiltration device (10,000 molecular weight cut-off).

The sample was dialysed against ultra high quality water and lyophilised. After resuspension in loading buffer, the proteins were separated by preparative 2-Dimensional-Gel Electrophoresis. Following electrophoresis an individual spot was chosen for study. The spot was subjected to in-gel tryptic digestion. The resulting peptides were extracted from the gel and purified using microbore RP-HPLC. Fractions were collected every 45 seconds and a portion of these consistent with the regions of UV absorbance were analysed by Delayed Extraction-Matrix Assisted Laser Desorption-Time of Flight Mass Spectrometry (DE-MALDI-TOF-MS). Peptides not observed in a blank preparation were then subjected to sequencing using Nanospray-MS/MS

Using this peptide sequence information, degenerate oligonucleotides were designed to be used in a polymerase chain reaction (PCR) to amplify the DNA segment lying between the peptide sequences identified.

PCR amplification resulted in the production of several polynucleotide fragments, each of which was cloned into the pCR 2.1-TOPO vector (Invitrogen BV, Netherlands) according to manufacturers protocol.

The DNA fragment in each plasmid was identified by sequencing and then used to obtain the full-length gene sequence, as follows.

Using the identified DNA fragment, oligonucleotide primers were designed for genomic DNA sequencing. These primers were designed so as to sequence in an `outward' direction from the obtained sequence. Once read, the sequence obtained was checked to see if the 5' and 3' termini of the gene had been reached. The presence of these features was identified by checking against homologous sequences, and for the 5' end the presence of an AUG start codon (or accepted alternative) preceded by a Shine-Dalgarno consensus sequence, and for the 3' end, the presence of a translation termination (Stop) codon.

Upon identification of the full-length gene, primers were designed for amplification of full-length product from GBS genomic DNA. Primers used included restriction enzyme recognition sites (NcoI at the 5'end and Eco0109I at the 3' end) to allow subsequent cloning of the product into the Lactococcal expression system used.

PCR was carried out using the primers, and the products cloned into a pCR 2.1 cloning vector (In Vitrogen). Following confirmation of the presence of the cloned fragment, the DNA was excised using the restriction enzymes NcoI and Eco0109I.

The vector into which this fragment was inserted was a modified version of pNZ8048 (Kuipers, O. P. *et al.* (1998) J. Biotech 64: 15-21). This vector, harbouring a lactococcal origin of replication, a chloramphenicol resistance marker, an inducible nisin promoter and a multicloning site was altered by the replacement of the multicloning site with two 10X His tags, flanked on the 5-most end with an NcoI site, split in the middle with a multicloning site (including an Eco0109I site), and a Stop (termination) codon at the 3'end of the His tags.

The gene of interest was inserted so that a 10X His tag was in the 3' position relative to the coding region.

Following transformation of the recombinant plasmid into *L.lactis* (strain NZ9000 - Kuipers, O. P. *et al.* (1998) *supra)*, a 400 ml liquid culture was set up and translation of the protein was induced by the addition of nisin to the culture. After a 2 hour incubation, the cells were harvested and lysed by bead beating. The resultant lysate was cleared by centrifugation, then passed over a metal affinity (Talon, Clonetech) column. The column was washed repeatedly before bound proteins were eluted with Imidazole.

To identify fractions containing the His-tagged recombinant protein, an aliquot from each fraction was analysed by SDS-PAGE, Western blotted and probed with anti-His antibodies.

The recombinant protein obtained was then used to immunise New Zealand white rabbits, with pre-immune sera being harvested prior to immunisation. Following a boost, the rabbits were sacrificed and sera collected. This sera was used in Western blots, ELISA and animal protection models.

Using the sera obtained from the animal studies, immunosorption studies were carried out.

Group B *Streptococcus* was grown in 20ml Todd Hewitt broth (THB) for 8 hours, harvested and resuspended in 5ml PBS. 50μl aliquots of this were used to coat wells in a 96 well plate (Nunc Immuno-Sorb). This was left at 4°C overnight to allow for absorbance of the bacteria onto the plate. Plates were washed twice with PBS, then blocked with 3%BSA in PBS for 1hr at 37°C. Plates were again washed. Serial 10 fold dilutions of the sera were made in PBS and 50μl of these dilutions were added to the wells of the plate, in duplicate. The plate was covered and incubated for 1 hr at 37°C. The plate was washed, then 50μl anti-rabbit alkaline phosphatase conjugated secondary antibody at a concentration of 1:5000 was added to each well. Following incubation at 37°C for an hour, the plate was washed again. 50μl substrate (PNPP) was added to each

well, and the reaction allowed to proceed for 30min before the absorbance was read at 405 nm.

Animal protection studies were also carried out to test the effectiveness of protection on the immunised rabbits.

GBS M732 was grown up in THB until mid-log phase was reached - approximately 5 hours. Cells were counted in a counting chamber, and bacteria were diluted to give a concentration of $2x10^7$ bacteria per ml in pre-immune or test sera. 50μl of this was injected via the intraperitoneal route into 0-1 day old mice. The mice were observed for survival over 48 hours.

The following Examples illustrate the invention.

Example 1

A first plasmid was termed MS4. The cloned DNA fragment was sequenced and the nucleotide and deduced amino acid sequence (SEQ ID NO. 1 and 2) was used to search protein databases.

Homologues to the GBS MS4 gene product can be identified in *Clostridium perfingens*, *Haemophilus influenzae*, *Neisseria flavescens* and *Thermatoga maritima*. In all cases the homologues are the genes for Ornithine Carbamoyltransferase (OCT). In eukaryotic systems this enzyme catalyses the second step in the Urea cycle, the conversion of ornithine to citrulline, a reaction requiring carbomyl phosphate. In prokaryotes, ODC is one of the three enzymes involved in Arginine Deaminase activity - a system which protects bacteria from acid damage. In particular, ODC is responsible for the conversion of citrulline to ornithine and carbamoyl phosphate (the opposite role to that in eukaryotes) (Casiano-Colon, A and Marquis, R. E. 1988. Appl. Environ. Microbiol. 54: 1318-1324, Cunin, R. *et al*. 1986. Microbiol. Rev. 50: 314-352).

Animal protection studies were carried out as described above. The results are as follows:

Treatment	# pups	# pups surviving at time (hrs)	
		24	48
PBS	15	6	0
Pre-Immune	41	18	1
Test	41	33	14

Example 2

A second plasmid was termed MS11. The nucleotide and deduced amino acid sequence (SEQ ID NOS. 3 and 4) were used to search protein databases.

Homologues to the GBS MS11 gene product can be identified in *Lactobacillus delbrueckii, Thermotoga maritima, Clostridium acetobulylicum, Bacillus megaterium, Triticum aestivium and Synechocystis PCC6803*.

In all cases the homologues are the genes for the protein Phosphoglycerate Kinase (PGK). PGK is a major enzyme in the glycolytic pathway, being involved in the conversion of Glyceraldehyde-3-phosphate to Phosphoenolpyruvate. In particular, it is involved in the catalysis of the reaction between Glycerate-1,3-diphosphate and 3-Phospho-Glycerate, releasing a phosphate in the forward reaction.

Example 3

A third plasmid was termed pMS16. The 5' and 3' cloned DNA fragments were sequenced and the nucleotide and deduced amino acid sequences for each are shown as SEQ ID NOS. 5 and 6 for the 5' fragment and SEQ ID NOS. 7 and 8 for the 3' fragment.

Homologues to the GBS MS16 gene product can be identified in *Bacillus stearothermophilus, Bacillus subtilis* and *Mycoplasma genitalium*.

In all cases the homologues are the genes for the protein Glucose-6-Phosphate Isomerase (GPI).

The enzyme Glucose-6-Phospate Isomerase catalyses the reaction between Glucose-6-phosphate and Fructose-6-Phosphate in both glycolysis (G6P to F6P) and

gluconeogenesis (F6P to G6P). Mutations in the *gpi* gene have been shown to confer purine analogue sensitivity to organisms.

Example 4

A fourth plasmid was termed pMS14. The cloned DNA fragment was sequenced and the nucleotide and deduced amino acid sequence (SEQ ID NOS. 9 and 10) was used to search protein databases.

Homologues to the GBS MS14 gene product can be identified in *Bacillus subtilis*, *Bacillus stearothermophilus*, *Mus musculus*, *Bos taurus* and *Zea mays*. In all cases the homologues are the genes for the protein Purine Nucleoside Phosphatase (PNP). The function of this enzyme is to cleave the nucleosides guanosine or inosine to their respective basis and sugar-1-phosphate molecules in the presence of orthophosphate.

Example 5

A fifth plasmid was termed pMS10. The cloned DNA fragment was sequenced. The nucleotide and deduced amino acid sequence (SEQ ID NOS. 11 and 12) was used to search protein databases.

Homologues to the GBS MS10 gene product can be identified in *Streptococcus mutans*, *Nicotiana plumb*, *Pisum sativum* and *Zea mays*. In all cases the homologues are the genes for the protein Nonphosphorylating, NADP-Dependent Glyceraldehyde-3-Phosphate Dehydrogenase (NPGAP-3-DH). NPGAP-3-DH has been reported as being an important means of generating NADPH for biosynthetic reactions in *S. mutans* (as opposed to NAD-specific GAP-3-DH which satisfies the requirements of the glycolytic pathway) (Boyd, D.A., Cvitkovitch, D. G. and Hamilton, I. R 1995 J. Bacteriol. 177: 2622-2727).

CLAIMS

1. A peptide encoded by an operon including any of the genes identified herein as MS4, MS10, MS11, MS14 and MS16, obtainable from Group B *streptococcus*, or a homologue thereof or a functional fragment thereof.

2. A peptide according to claim 1, comprising any of the amino acid sequences identified herein as SEQ ID NOS. 2, 4, 6, 8, 10 and 12.

3. A peptide according to claim 1 or claim 2, for therapeutic use.

4. A polynucleotide encoding a peptide according to claim 1 or claim 2, for therapeutic use.

5. A host transformed to express a peptide according to claim 1 or claim 2.

6. A vaccine comprising a peptide according to claim 1 or claim 2, or the means for its expression.

7. Use of a product according to any of claims 1 to 5, for screening potential drugs or for the detection of virulence.

8. Use of a product according to any of claims 1 to 5, for the manufacture of a medicament for use in the treatment or prevention of a condition associated with bacterial infection.

9. Use according to claim 8, wherein the infection is a Group B *streptococcal* infection.

10. Use according to claim 8 or claim 9, wherein the infection is a focal infection.

11. Use according to claim 8 or claim 9, wherein the infection is a urinary tract infection.

12. An antibody raised against a peptide according to claim 1 or claim 2.

Abstract of the Disclosure

According to the present invention, a series of genes are identified in Group B *Streptococcus*, the products of which may be located on the outer surface of the organism. The genes, or functional fragments thereof, may be useful in the preparation of therapeutics, *e.g.* vaccines for the immunization of a patient against microbial infection.

SEQUENCE LISTING

<110> Microscience Limited

<120> OUTER SURFACE PROTEINS, THEIR GENES, AND THEIR USE

<130> REP05969WO

<140>
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<160> 12

<170> PatentIn Ver. 2.1

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Met Thr Gln Val Phe Gln Gly Arg Ser Phe Leu Ala Glu Lys Asp Phe
  1               5                  10                  15

tct cgt gag gaa ttt gaa tat ctt att gat ttt tca gct cat tta aaa   96
Ser Arg Glu Glu Phe Glu Tyr Leu Ile Asp Phe Ser Ala His Leu Lys
             20                  25                  30

gac ctt aaa aaa cgt ggt gtt cct cat cat tat ctt gaa ggt aaa aat   144
Asp Leu Lys Lys Arg Gly Val Pro His His Tyr Leu Glu Gly Lys Asn
         35                  40                  45

att gct ctc tta ttt gaa aaa aca tct act cgt act cgc gca gcc ttt   192
Ile Ala Leu Leu Phe Glu Lys Thr Ser Thr Arg Thr Arg Ala Ala Phe
     50                  55                  60

aca act gca gca att gac cta ggc gct cat ccg gaa tac ctt ggt gca   240
Thr Thr Ala Ala Ile Asp Leu Gly Ala His Pro Glu Tyr Leu Gly Ala
 65                  70                  75                  80

aat gat att caa ctt ggt aaa aaa gaa tca aca gaa gat act gct aag   288
Asn Asp Ile Gln Leu Gly Lys Lys Glu Ser Thr Glu Asp Thr Ala Lys
                 85                  90                  95
```

```
gtt tta gga cgt atg ttt gat ggt att gaa ttc cgt ggt ttt agc caa   336
Val Leu Gly Arg Met Phe Asp Gly Ile Glu Phe Arg Gly Phe Ser Gln
            100                 105                 110

aga atg gtt gaa gag ctt gct gaa ttt tct gga gta cct gtc tgg aat   384
Arg Met Val Glu Glu Leu Ala Glu Phe Ser Gly Val Pro Val Trp Asn
        115                 120                 125

ggt tta aca gat gaa tgg cat cca aca caa atg cta gct gac tac ctt   432
Gly Leu Thr Asp Glu Trp His Pro Thr Gln Met Leu Ala Asp Tyr Leu
    130                 135                 140

act atc aaa gaa aac ttc ggt aaa ctt gaa ggt att act ctt gtt tac   480
Thr Ile Lys Glu Asn Phe Gly Lys Leu Glu Gly Ile Thr Leu Val Tyr
145                 150                 155                 160

tgt ggt gac gga cgt aac aat gtt gcc aac tcg ctt tta gtg gct ggg   528
Cys Gly Asp Gly Arg Asn Asn Val Ala Asn Ser Leu Leu Val Ala Gly
                165                 170                 175

act ttg atg ggg gtc aat gta cac atc ttt tct cca aaa gaa ctt tty   576
Thr Leu Met Gly Val Asn Val His Ile Phe Ser Pro Lys Glu Leu Phe
            180                 185                 190

ccw gct gaa gag att gtt aaa ttg gct gaa gga tat gcc aaa gaa tct   624
Xaa Ala Glu Glu Ile Val Lys Leu Ala Glu Gly Tyr Ala Lys Glu Ser
        195                 200                 205

ggg gct cac gtt ctc gtt act gat aat gta gac gaa gct gta aag gga   672
Gly Ala His Val Leu Val Thr Asp Asn Val Asp Glu Ala Val Lys Gly
    210                 215                 220

gca gac gtc ttt tac act gat gtc tgg gta tcg atg gga gaa gaa gat   720
Ala Asp Val Phe Tyr Thr Asp Val Trp Val Ser Met Gly Glu Glu Asp
225                 230                 235                 240

aag ttc aaa gaa cgc gtt gaa ctt ctt caa cca tat caa gta aac atg   768
Lys Phe Lys Glu Arg Val Glu Leu Leu Gln Pro Tyr Gln Val Asn Met
                245                 250                 255

gaa ctg att aaa aaa gct aat aat gat aat ctt atc ttc tta cac tgc   816
Glu Leu Ile Lys Lys Ala Asn Asn Asp Asn Leu Ile Phe Leu His Cys
            260                 265                 270

tta cct gca ttc cat gat aca aat acc gtt tat ggc aaa gac gtc gct   864
Leu Pro Ala Phe His Asp Thr Asn Thr Val Tyr Gly Lys Asp Val Ala
        275                 280                 285
```

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gaa aaa ttt ggg gtc aag gaa atg gaa gtt act gat gaa gtc ttc cgt    912
Glu Lys Phe Gly Val Lys Glu Met Glu Val Thr Asp Glu Val Phe Arg
    290                 295                 300

agc aaa tat gct cgt cat ttc gac caa gct gaa aat cgt atg cac act    960
Ser Lys Tyr Ala Arg His Phe Asp Gln Ala Glu Asn Arg Met His Thr
305                 310                 315                 320

att aaa gct gta atg gct gca acc ctt gga aat ctt ttc att cca aaa   1008
Ile Lys Ala Val Met Ala Ala Thr Leu Gly Asn Leu Phe Ile Pro Lys
                325                 330                 335

gtt taa                                                          1014
Val
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<210> 2
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<213> group B streptococcus

<400> 2

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Met Thr Gln Val Phe Gln Gly Arg Ser Phe Leu Ala Glu Lys Asp Phe
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Ser Arg Glu Glu Phe Glu Tyr Leu Ile Asp Phe Ser Ala His Leu Lys
             20                  25                  30

Asp Leu Lys Lys Arg Gly Val Pro His His Tyr Leu Glu Gly Lys Asn
         35                  40                  45

Ile Ala Leu Leu Phe Glu Lys Thr Ser Thr Arg Thr Arg Ala Ala Phe
     50                  55                  60

Thr Thr Ala Ala Ile Asp Leu Gly Ala His Pro Glu Tyr Leu Gly Ala
 65                  70                  75                  80

Asn Asp Ile Gln Leu Gly Lys Lys Glu Ser Thr Glu Asp Thr Ala Lys
                 85                  90                  95

Val Leu Gly Arg Met Phe Asp Gly Ile Glu Phe Arg Gly Phe Ser Gln
            100                 105                 110

Arg Met Val Glu Glu Leu Ala Glu Phe Ser Gly Val Pro Val Trp Asn
        115                 120                 125

Gly Leu Thr Asp Glu Trp His Pro Thr Gln Met Leu Ala Asp Tyr Leu
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130 135 140

Thr Ile Lys Glu Asn Phe Gly Lys Leu Glu Gly Ile Thr Leu Val Tyr
145 150 155 160

Cys Gly Asp Gly Arg Asn Asn Val Ala Asn Ser Leu Leu Val Ala Gly
165 170 175

Thr Leu Met Gly Val Asn Val His Ile Phe Ser Pro Lys Glu Leu Phe
180 185 190

Xaa Ala Glu Glu Ile Val Lys Leu Ala Glu Gly Tyr Ala Lys Glu Ser
195 200 205

Gly Ala His Val Leu Val Thr Asp Asn Val Asp Glu Ala Val Lys Gly
210 215 220

Ala Asp Val Phe Tyr Thr Asp Val Trp Val Ser Met Gly Glu Glu Asp
225 230 235 240

Lys Phe Lys Glu Arg Val Glu Leu Leu Gln Pro Tyr Gln Val Asn Met
245 250 255

Glu Leu Ile Lys Lys Ala Asn Asn Asp Asn Leu Ile Phe Leu His Cys
260 265 270

Leu Pro Ala Phe His Asp Thr Asn Thr Val Tyr Gly Lys Asp Val Ala
275 280 285

Glu Lys Phe Gly Val Lys Glu Met Glu Val Thr Asp Glu Val Phe Arg
290 295 300

Ser Lys Tyr Ala Arg His Phe Asp Gln Ala Glu Asn Arg Met His Thr
305 310 315 320

Ile Lys Ala Val Met Ala Ala Thr Leu Gly Asn Leu Phe Ile Pro Lys
325 330 335

Val

<210> 3
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<212> DNA
<213> group B streptococcus

<220>

<221> CDS
<222> (1)..(1197)

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Met Ala Lys Leu Thr Val Lys Asp Val Asp Leu Lys Val Lys Lys Val
  1               5                  10                  15

ctc gtt cgt gtt gac ttt aat gtg cct ttg aaa gac ggc gtt atc act   96
Leu Val Arg Val Asp Phe Asn Val Pro Leu Lys Asp Gly Val Ile Thr
             20                  25                  30

aac gac aac cgt atc act gcg gct ctt cca aca atc aag tat atc atc   144
Asn Asp Asn Arg Ile Thr Ala Ala Leu Pro Thr Ile Lys Tyr Ile Ile
         35                  40                  45

gaa caa ggt ggt cgt gct atc ctc ttc tct cac ctt gga cgt gtt aaa   192
Glu Gln Gly Gly Arg Ala Ile Leu Phe Ser His Leu Gly Arg Val Lys
     50                  55                  60

gaa gaa gct gac aaa gaa gga aaa tca ctt gca ccg gta gct gct gat   240
Glu Glu Ala Asp Lys Glu Gly Lys Ser Leu Ala Pro Val Ala Ala Asp
 65                  70                  75                  80

tta gct gct aaa ctt ggt caa gat gtt gta ttc cca ggt gtt act cgt   288
Leu Ala Ala Lys Leu Gly Gln Asp Val Val Phe Pro Gly Val Thr Arg
                 85                  90                  95

ggt gca aaa tta gaa gaa gca atc aat gct ttg gaa gat gga caa gtt   336
Gly Ala Lys Leu Glu Glu Ala Ile Asn Ala Leu Glu Asp Gly Gln Val
            100                 105                 110

ctt ttg gtt gaa aac act cgt ttt gaa gat gtt gac ggt aag aaa gaa   384
Leu Leu Val Glu Asn Thr Arg Phe Glu Asp Val Asp Gly Lys Lys Glu
        115                 120                 125

tct aag aat gac gaa gaa ctt ggt aaa tac tgg gct tca ctt gga gat   432
Ser Lys Asn Asp Glu Glu Leu Gly Lys Tyr Trp Ala Ser Leu Gly Asp
    130                 135                 140

gga atc ttc gtt aac gat gca ttt ggt aca gca cac cgt gct cat gca   480
Gly Ile Phe Val Asn Asp Ala Phe Gly Thr Ala His Arg Ala His Ala
145                 150                 155                 160

tca aac gta ggt att tca gca aac gtt gaa aaa gct gta gct ggt ttc   528
Ser Asn Val Gly Ile Ser Ala Asn Val Glu Lys Ala Val Ala Gly Phe
                165                 170                 175
```

```
ctt ctt gaa aac gaa att gct tac atc caa gaa gca gtt gaa act cca   576
Leu Leu Glu Asn Glu Ile Ala Tyr Ile Gln Glu Ala Val Glu Thr Pro
            180                 185                 190

gaa cgc cca ttc gta gct att ctt ggt ggc tca aaa gtt tct gat aag   624
Glu Arg Pro Phe Val Ala Ile Leu Gly Gly Ser Lys Val Ser Asp Lys
        195                 200                 205

att ggt gtt atc gaa aac ctt ctt gaa aaa gct gat aaa gtt ctt atc   672
Ile Gly Val Ile Glu Asn Leu Leu Glu Lys Ala Asp Lys Val Leu Ile
    210                 215                 220

ggt ggt ggt atg act tac aca ttc tac aaa gct caa ggt atc gaa atc   720
Gly Gly Gly Met Thr Tyr Thr Phe Tyr Lys Ala Gln Gly Ile Glu Ile
225                 230                 235                 240

ggt aac tca ctt gta gaa gaa gac aaa ttg gat gtt gct aaa gac ctc   768
Gly Asn Ser Leu Val Glu Glu Asp Lys Leu Asp Val Ala Lys Asp Leu
                245                 250                 255

ctt gaa aaa tca aac ggt aaa ttg atc ttg cca gtt gac tca aaa gaa   816
Leu Glu Lys Ser Asn Gly Lys Leu Ile Leu Pro Val Asp Ser Lys Glu
            260                 265                 270

gca aac gca ttt gct ggt tat act gaa gtt cgc gac act gaa ggt gaa   864
Ala Asn Ala Phe Ala Gly Tyr Thr Glu Val Arg Asp Thr Glu Gly Glu
        275                 280                 285

gca gtt tca gaa ggg ttc ctt ggt ctt gac atc ggt cct aaa tca atc   912
Ala Val Ser Glu Gly Phe Leu Gly Leu Asp Ile Gly Pro Lys Ser Ile
    290                 295                 300

gct aaa ttt gat gaa gca ctt act ggt gct aaa aca gtt gta tgg aac   960
Ala Lys Phe Asp Glu Ala Leu Thr Gly Ala Lys Thr Val Val Trp Asn
305                 310                 315                 320

gga cct atg ggt gtc ttt gaa aac cct gac ttc caa gct ggt aca atc   1008
Gly Pro Met Gly Val Phe Glu Asn Pro Asp Phe Gln Ala Gly Thr Ile
                325                 330                 335

ggt gta atg gac gct atc gtt aaa caa cca ggc gtt aaa tca atc atc   1056
Gly Val Met Asp Ala Ile Val Lys Gln Pro Gly Val Lys Ser Ile Ile
            340                 345                 350

ggt ggt ggt gat tca gca gca gct gct atc aac ctt ggt cgt gct gac   1104
Gly Gly Gly Asp Ser Ala Ala Ala Ala Ile Asn Leu Gly Arg Ala Asp
        355                 360                 365
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aaa ttc tca tgg atc tct act ggt ggt gga gca agc atg gaa ttg ctc   1152
Lys Phe Ser Trp Ile Ser Thr Gly Gly Gly Ala Ser Met Glu Leu Leu
    370                 375                 380

gaa ggt aaa gta tta cca ggt ttg gca gca ttg act gaa aaa taa       1197
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385                 390                 395
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<210> 4
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<212> PRT
<213> group B streptococcus

<400> 4

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            20                  25                  30

Asn Asp Asn Arg Ile Thr Ala Ala Leu Pro Thr Ile Lys Tyr Ile Ile
        35                  40                  45

Glu Gln Gly Gly Arg Ala Ile Leu Phe Ser His Leu Gly Arg Val Lys
    50                  55                  60

Glu Glu Ala Asp Lys Glu Gly Lys Ser Leu Ala Pro Val Ala Ala Asp
65                  70                  75                  80

Leu Ala Ala Lys Leu Gly Gln Asp Val Val Phe Pro Gly Val Thr Arg
                85                  90                  95

Gly Ala Lys Leu Glu Glu Ala Ile Asn Ala Leu Glu Asp Gly Gln Val
            100                 105                 110

Leu Leu Val Glu Asn Thr Arg Phe Glu Asp Val Asp Gly Lys Lys Glu
        115                 120                 125

Ser Lys Asn Asp Glu Glu Leu Gly Lys Tyr Trp Ala Ser Leu Gly Asp
    130                 135                 140

Gly Ile Phe Val Asn Asp Ala Phe Gly Thr Ala His Arg Ala His Ala
145                 150                 155                 160

Ser Asn Val Gly Ile Ser Ala Asn Val Glu Lys Ala Val Ala Gly Phe
                165                 170                 175
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```
Leu Leu Glu Asn Glu Ile Ala Tyr Ile Gln Glu Ala Val Glu Thr Pro
            180                 185                 190

Glu Arg Pro Phe Val Ala Ile Leu Gly Gly Ser Lys Val Ser Asp Lys
        195                 200                 205

Ile Gly Val Ile Glu Asn Leu Leu Glu Lys Ala Asp Lys Val Leu Ile
    210                 215                 220

Gly Gly Gly Met Thr Tyr Thr Phe Tyr Lys Ala Gln Gly Ile Glu Ile
225                 230                 235                 240

Gly Asn Ser Leu Val Glu Glu Asp Lys Leu Asp Val Ala Lys Asp Leu
                245                 250                 255

Leu Glu Lys Ser Asn Gly Lys Leu Ile Leu Pro Val Asp Ser Lys Glu
            260                 265                 270

Ala Asn Ala Phe Ala Gly Tyr Thr Glu Val Arg Asp Thr Glu Gly Glu
        275                 280                 285

Ala Val Ser Glu Gly Phe Leu Gly Leu Asp Ile Gly Pro Lys Ser Ile
    290                 295                 300

Ala Lys Phe Asp Glu Ala Leu Thr Gly Ala Lys Thr Val Val Trp Asn
305                 310                 315                 320

Gly Pro Met Gly Val Phe Glu Asn Pro Asp Phe Gln Ala Gly Thr Ile
                325                 330                 335

Gly Val Met Asp Ala Ile Val Lys Gln Pro Gly Val Lys Ser Ile Ile
            340                 345                 350

Gly Gly Gly Asp Ser Ala Ala Ala Ala Ile Asn Leu Gly Arg Ala Asp
        355                 360                 365

Lys Phe Ser Trp Ile Ser Thr Gly Gly Gly Ala Ser Met Glu Leu Leu
    370                 375                 380

Glu Gly Lys Val Leu Pro Gly Leu Ala Ala Leu Thr Glu Lys
385                 390                 395
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<210> 5
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<212> DNA
<213> group B streptococcus

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<221> CDS
<222> (1)..(516)

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Met Thr His Ile Thr Phe Asp Leu Phe Lys Val Leu Gly Gln Phe Val
  1               5                  10                  15

ggc gaa cac gag tta gac tac cta cca cca caa gta agt gca gca gat   96
Gly Glu His Glu Leu Asp Tyr Leu Pro Pro Gln Val Ser Ala Ala Asp
             20                  25                  30

gct ttc ctt cgt caa ggg act ggt cct gga tca gat ttt ctc gga tgg   144
Ala Phe Leu Arg Gln Gly Thr Gly Pro Gly Ser Asp Phe Leu Gly Trp
         35                  40                  45

atg gaa cct cca gaa aac tat gac aaa gaa gaa ttt tct cgc att caa   192
Met Glu Pro Pro Glu Asn Tyr Asp Lys Glu Glu Phe Ser Arg Ile Gln
     50                  55                  60

aaa gcc gct gaa aag att aaa tca gat agc gaa gta ctc gtg gtt att   240
Lys Ala Ala Glu Lys Ile Lys Ser Asp Ser Glu Val Leu Val Val Ile
 65                  70                  75                  80

ggt att ggt ggt tcg tac ctt ggt gca aaa gca gca att gac ttt ttg   288
Gly Ile Gly Gly Ser Tyr Leu Gly Ala Lys Ala Ala Ile Asp Phe Leu
                 85                  90                  95

aat aat cat ttt gct aat ttg caa acc gca gaa gaa cgt aaa gcg cct   336
Asn Asn His Phe Ala Asn Leu Gln Thr Ala Glu Glu Arg Lys Ala Pro
            100                 105                 110

cag att ctt tat gct gga aat tct att tca tct act tac ctt gcc gat   384
Gln Ile Leu Tyr Ala Gly Asn Ser Ile Ser Ser Thr Tyr Leu Ala Asp
        115                 120                 125

tta gtt gaa tac gtc caa gat aaa gaa ttc tca gta aat gtc att tca   432
Leu Val Glu Tyr Val Gln Asp Lys Glu Phe Ser Val Asn Val Ile Ser
    130                 135                 140

aaa tca ggt aca aca act gaa cca gcg att gct ttc cgt gta ttt aaa   480
Lys Ser Gly Thr Thr Thr Glu Pro Ala Ile Ala Phe Arg Val Phe Lys
145                 150                 155                 160

gaa ctt cta gtt aaa aag tac cgg tca aga aga agc                   516
Glu Leu Leu Val Lys Lys Tyr Arg Ser Arg Arg Ser
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165 170

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<213> group B streptococcus

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20 25 30

Ala Phe Leu Arg Gln Gly Thr Gly Pro Gly Ser Asp Phe Leu Gly Trp
35 40 45

Met Glu Pro Pro Glu Asn Tyr Asp Lys Glu Glu Phe Ser Arg Ile Gln
50 55 60

Lys Ala Ala Glu Lys Ile Lys Ser Asp Ser Glu Val Leu Val Val Ile
65 70 75 80

Gly Ile Gly Gly Ser Tyr Leu Gly Ala Lys Ala Ala Ile Asp Phe Leu
85 90 95

Asn Asn His Phe Ala Asn Leu Gln Thr Ala Glu Glu Arg Lys Ala Pro
100 105 110

Gln Ile Leu Tyr Ala Gly Asn Ser Ile Ser Ser Thr Tyr Leu Ala Asp
115 120 125

Leu Val Glu Tyr Val Gln Asp Lys Glu Phe Ser Val Asn Val Ile Ser
130 135 140

Lys Ser Gly Thr Thr Thr Glu Pro Ala Ile Ala Phe Arg Val Phe Lys
145 150 155 160

Glu Leu Leu Val Lys Lys Tyr Arg Ser Arg Arg Ser
165 170

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<213> group B streptococcus

<220>
<221> CDS
<222> (1)..(318)

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Ile Asn Arg Arg Phe Arg Trp Ser Trp Leu Ser Ser Arg Lys Asp Val
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gat ttt gtt aat aaa aaa gca aca gat ggt gtg ctt ctt gct cat aca 96
Asp Phe Val Asn Lys Lys Ala Thr Asp Gly Val Leu Leu Ala His Thr
20 25 30

gat ggt ggg gtt cca aat atg ttt gta acg ctt cct aca caa gac gct 144
Asp Gly Gly Val Pro Asn Met Phe Val Thr Leu Pro Thr Gln Asp Ala
35 40 45

tac act ctt ggt tac act att tac ttc ttt gag tta gca att ggc ctt 192
Tyr Thr Leu Gly Tyr Thr Ile Tyr Phe Phe Glu Leu Ala Ile Gly Leu
50 55 60

tca ggt tat ctt aac tca gta aat cca ttt gat caa ccg ggg gta gaa 240
Ser Gly Tyr Leu Asn Ser Val Asn Pro Phe Asp Gln Pro Gly Val Glu
65 70 75 80

gca tat aaa cgt aat atg ttc gca ttt ggt aaa cct gga ttc gaa gag 288
Ala Tyr Lys Arg Asn Met Phe Ala Phe Gly Lys Pro Gly Phe Glu Glu
85 90 95

ctt agc gct gaa ttg aat gca cgt ctt taa 318
Leu Ser Ala Glu Leu Asn Ala Arg Leu
100 105

<210> 8
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<212> PRT
<213> group B streptococcus

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Ile Asn Arg Arg Phe Arg Trp Ser Trp Leu Ser Ser Arg Lys Asp Val
1 5 10 15

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20 25 30

Asp Gly Gly Val Pro Asn Met Phe Val Thr Leu Pro Thr Gln Asp Ala

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         35                  40                  45

Tyr Thr Leu Gly Tyr Thr Ile Tyr Phe Phe Glu Leu Ala Ile Gly Leu
     50                  55                  60

Ser Gly Tyr Leu Asn Ser Val Asn Pro Phe Asp Gln Pro Gly Val Glu
 65                  70                  75                  80

Ala Tyr Lys Arg Asn Met Phe Ala Phe Gly Lys Pro Gly Phe Glu Glu
                 85                  90                  95

Leu Ser Ala Glu Leu Asn Ala Arg Leu
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<220>
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  1               5                  10                  15

aaa ggc gtc aca gca cca gaa ttt ggy ctt att tta ggc tct ggt tta   96
Lys Gly Val Thr Ala Pro Glu Phe Xaa Leu Ile Leu Gly Ser Gly Leu
             20                  25                  30

gga gaa ttg gct gaa gaa atc gaa aat cct att gtt gtg gat tat gca   144
Gly Glu Leu Ala Glu Glu Ile Glu Asn Pro Ile Val Val Asp Tyr Ala
         35                  40                  45

gac atc ccm aat tgg gga cag tca aca gta gtt ggt cat gct gga aaa   192
Asp Ile Xaa Asn Trp Gly Gln Ser Thr Val Val Gly His Ala Gly Lys
     50                  55                  60

ttt agt gta tgg gat tta tca ggc cgt aag gta tta gcg ctt caa ggt   240
Phe Ser Val Trp Asp Leu Ser Gly Arg Lys Val Leu Ala Leu Gln Gly
 65                  70                  75                  80

cgt ttt cat ttt tay gaa ggw aat aca atg gaa gtc gtt act ttc cca   288
Arg Phe His Phe Tyr Glu Xaa Asn Thr Met Glu Val Val Thr Phe Pro
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                85                  90                  95

gta cgt atc atg aga gca ttg gct tgc cac agt gtg ctt gtg act aat   336
Val Arg Ile Met Arg Ala Leu Ala Cys His Ser Val Leu Val Thr Asn
            100                 105                 110

gca gcg ggt ggg att gga tac gga cca gga act tta atg ctg atc aaa   384
Ala Ala Gly Gly Ile Gly Tyr Gly Pro Gly Thr Leu Met Leu Ile Lys
        115                 120                 125

gac cac atc aat atg att ggg act aac cct ctc ata ggt gag aac ctt   432
Asp His Ile Asn Met Ile Gly Thr Asn Pro Leu Ile Gly Glu Asn Leu
    130                 135                 140

gaa gaa ttt gga cca cgt ttc cca gac atg tcg gat gct tay aca gca   480
Glu Glu Phe Gly Pro Arg Phe Pro Asp Met Ser Asp Ala Tyr Thr Ala
145                 150                 155                 160

aca tat cga caa aaa gct cac caa att gct gaa aac gat atc aaa ctc   528
Thr Tyr Arg Gln Lys Ala His Gln Ile Ala Glu Asn Asp Ile Lys Leu
                165                 170                 175

gaa gaa ggt gtg tac ttg ggt gta tca gga ccc act tat gaa aca cct   576
Glu Glu Gly Val Tyr Leu Gly Val Ser Gly Pro Thr Tyr Glu Thr Pro
            180                 185                 190

gca gaa att cgt gca ttc caa aca atg ggc gca caa gcg gta ggt atg   624
Ala Glu Ile Arg Ala Phe Gln Thr Met Gly Ala Gln Ala Val Gly Met
        195                 200                 205

tcc acg gtt cca gag gtg atc gtt gca gct cac tca ggg ctt aaa gtg   672
Ser Thr Val Pro Glu Val Ile Val Ala Ala His Ser Gly Leu Lys Val
    210                 215                 220

tta gga att tca gca att act aac ctt gcc gct ggc ttc caa tca gag   720
Leu Gly Ile Ser Ala Ile Thr Asn Leu Ala Ala Gly Phe Gln Ser Glu
225                 230                 235                 240

ctc aat cat gag gag gtc gtt gaa gtt act cag cgt att aaa gaa gat   768
Leu Asn His Glu Glu Val Val Glu Val Thr Gln Arg Ile Lys Glu Asp
                245                 250                 255

ttc aag gga tta ggt aaa tca tta gtt gct gaa ctc                   804
Phe Lys Gly Leu Gly Lys Ser Leu Val Ala Glu Leu
            260                 265
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<212> PRT
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            20                  25                  30

Gly Glu Leu Ala Glu Glu Ile Glu Asn Pro Ile Val Val Asp Tyr Ala
        35                  40                  45

Asp Ile Xaa Asn Trp Gly Gln Ser Thr Val Val Gly His Ala Gly Lys
    50                  55                  60

Phe Ser Val Trp Asp Leu Ser Gly Arg Lys Val Leu Ala Leu Gln Gly
65                  70                  75                  80

Arg Phe His Phe Tyr Glu Xaa Asn Thr Met Glu Val Val Thr Phe Pro
                85                  90                  95

Val Arg Ile Met Arg Ala Leu Ala Cys His Ser Val Leu Val Thr Asn
            100                 105                 110

Ala Ala Gly Gly Ile Gly Tyr Gly Pro Gly Thr Leu Met Leu Ile Lys
        115                 120                 125

Asp His Ile Asn Met Ile Gly Thr Asn Pro Leu Ile Gly Glu Asn Leu
    130                 135                 140

Glu Glu Phe Gly Pro Arg Phe Pro Asp Met Ser Asp Ala Tyr Thr Ala
145                 150                 155                 160

Thr Tyr Arg Gln Lys Ala His Gln Ile Ala Glu Asn Asp Ile Lys Leu
                165                 170                 175

Glu Glu Gly Val Tyr Leu Gly Val Ser Gly Pro Thr Tyr Glu Thr Pro
            180                 185                 190

Ala Glu Ile Arg Ala Phe Gln Thr Met Gly Ala Gln Ala Val Gly Met
        195                 200                 205

Ser Thr Val Pro Glu Val Ile Val Ala Ala His Ser Gly Leu Lys Val
    210                 215                 220

Leu Gly Ile Ser Ala Ile Thr Asn Leu Ala Ala Gly Phe Gln Ser Glu
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Leu Asn His Glu Glu Val Val Glu Val Thr Gln Arg Ile Lys Glu Asp
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gtt aat cag att gag att ttg tca cca att gat gat tct tca ttg gga 96
Val Asn Gln Ile Glu Ile Leu Ser Pro Ile Asp Asp Ser Ser Leu Gly
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Phe Val Pro Ala Met Thr Arg Glu Glu Val Asp His Ala Met Lys Ala
35 40 45

ggt cgt gag gct tta cca gct tgg gct gct tta aca gta tat gaa cgt 192
Gly Arg Glu Ala Leu Pro Ala Trp Ala Ala Leu Thr Val Tyr Glu Arg
50 55 60

gca caa tac ctt cat aaa gcc gca gac att att gaa cgt gat aaa gaa 240
Ala Gln Tyr Leu His Lys Ala Ala Asp Ile Ile Glu Arg Asp Lys Glu
65 70 75 80

gaa att gct act gtt tta gca aaa gaa att tct aaa gct tac aat gct 288
Glu Ile Ala Thr Val Leu Ala Lys Glu Ile Ser Lys Ala Tyr Asn Ala
85 90 95

tca gta act gag gtt gta agg aca gct gat ctt att cgt tat gca gca 336
Ser Val Thr Glu Val Val Arg Thr Ala Asp Leu Ile Arg Tyr Ala Ala
100 105 110

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Glu Glu Gly Ile Arg Leu Ser Thr Ser Ala Asp Glu Gly Gly Lys Met
        115                 120                 125

gat gct tca aca ggt cat aag ttg gct gtt att cgt cgt caa cca gta   432
Asp Ala Ser Thr Gly His Lys Leu Ala Val Ile Arg Arg Gln Pro Val
    130                 135                 140

ggt atc gtt tta gca atc gca cct tat aat tac cct gtt aac ctc tca   480
Gly Ile Val Leu Ala Ile Ala Pro Tyr Asn Tyr Pro Val Asn Leu Ser
145                 150                 155                 160

gga tca aaa att gcg cca gct cta att ggt gga aac gtt gtg atg ttt   528
Gly Ser Lys Ile Ala Pro Ala Leu Ile Gly Gly Asn Val Val Met Phe
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aaa cca cca aca caa ggt tca gtc tca gga ctt gtt tta gca aaa gct   576
Lys Pro Pro Thr Gln Gly Ser Val Ser Gly Leu Val Leu Ala Lys Ala
            180                 185                 190

ttt gca gaa gca ggt ctt cca gca ggt gtc ttt aat act att aca gga   624
Phe Ala Glu Ala Gly Leu Pro Ala Gly Val Phe Asn Thr Ile Thr Gly
        195                 200                 205

cgc ggt tct gag att gga gat tac att gtt gag cat gaa gaa gtt aat   672
Arg Gly Ser Glu Ile Gly Asp Tyr Ile Val Glu His Glu Glu Val Asn
    210                 215                 220

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Phe Ile Asn Phe Thr Gly Ser Thr Pro Val Gly Gln Arg Ile Gly Lys
225                 230                 235                 240

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Leu Ala Gly Met Arg Pro Ile Met Leu Glu Leu Gly Gly Lys Asp Ala
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Gly Ile Val Leu Ala Asp Ala Asp Leu Asp Asn Ala Ala Lys Gln Ile
            260                 265                 270

gtt gca ggt gct tat gat tac tct gga caa cgc tgt acg gca att aag   864
Val Ala Gly Ala Tyr Asp Tyr Ser Gly Gln Arg Cys Thr Ala Ile Lys
        275                 280                 285

cgt gtg ctt gtc gtt gaa gaa gtt gcw gat gaa ttg gca gaa aaa ata   912
Arg Val Leu Val Val Glu Glu Val Xaa Asp Glu Leu Ala Glu Lys Ile
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acg gtg aca ccg gtt att gat gat aat tca gct gac ttt att gaa agc   1008
Thr Val Thr Pro Val Ile Asp Asp Asn Ser Ala Asp Phe Ile Glu Ser
                325                 330                 335

tta gta gta gat gca cgt caa aaa ggt gcg aaa gaa ttg aat gaa ttt   1056
Leu Val Val Asp Ala Arg Gln Lys Gly Ala Lys Glu Leu Asn Glu Phe
            340                 345                 350

aaa cgt gat ggt cgt cta tta act cca gga ttg ttt gat cat gtt act   1104
Lys Arg Asp Gly Arg Leu Leu Thr Pro Gly Leu Phe Asp His Val Thr
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tta gat atg aaa cta gct tgg gaa gag cct ttt gga cca att ctc cca   1152
Leu Asp Met Lys Leu Ala Trp Glu Glu Pro Phe Gly Pro Ile Leu Pro
    370                 375                 380

att att cgt gtc aag gat gca gaa gaa gct gtt gct att gcc aac aaa   1200
Ile Ile Arg Val Lys Asp Ala Glu Glu Ala Val Ala Ile Ala Asn Lys
385                 390                 395                 400

tct gat ttt gga tta caa tca tca gtc ttt aca cgt gat ttc caa aaa   1248
Ser Asp Phe Gly Leu Gln Ser Ser Val Phe Thr Arg Asp Phe Gln Lys
                405                 410                 415

gca ttt gat ata gca aat aaa ctt gaa gtt ggt aca gtt cac att aac   1296
Ala Phe Asp Ile Ala Asn Lys Leu Glu Val Gly Thr Val His Ile Asn
            420                 425                 430

aat aag act gga cgt ggt ccw gat aat ttc cca ttc tta gga ctc aaa   1344
Asn Lys Thr Gly Arg Gly Xaa Asp Asn Phe Pro Phe Leu Gly Leu Lys
        435                 440                 445

gga tct ggt gca ggt gtt caa ggt atc aga tat tca att gaa gca atg   1392
Gly Ser Gly Ala Gly Val Gln Gly Ile Arg Tyr Ser Ile Glu Ala Met
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Phe Val Pro Ala Met Thr Arg Glu Glu Val Asp His Ala Met Lys Ala
         35                  40                  45

Gly Arg Glu Ala Leu Pro Ala Trp Ala Ala Leu Thr Val Tyr Glu Arg
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Ala Gln Tyr Leu His Lys Ala Ala Asp Ile Ile Glu Arg Asp Lys Glu
 65                  70                  75                  80

Glu Ile Ala Thr Val Leu Ala Lys Glu Ile Ser Lys Ala Tyr Asn Ala
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Ser Val Thr Glu Val Val Arg Thr Ala Asp Leu Ile Arg Tyr Ala Ala
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Glu Glu Gly Ile Arg Leu Ser Thr Ser Ala Asp Glu Gly Gly Lys Met
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Asp Ala Ser Thr Gly His Lys Leu Ala Val Ile Arg Arg Gln Pro Val
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Gly Ile Val Leu Ala Ile Ala Pro Tyr Asn Tyr Pro Val Asn Leu Ser
145                 150                 155                 160

Gly Ser Lys Ile Ala Pro Ala Leu Ile Gly Gly Asn Val Val Met Phe
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Lys Pro Pro Thr Gln Gly Ser Val Ser Gly Leu Val Leu Ala Lys Ala
            180                 185                 190

Phe Ala Glu Ala Gly Leu Pro Ala Gly Val Phe Asn Thr Ile Thr Gly
        195                 200                 205

Arg Gly Ser Glu Ile Gly Asp Tyr Ile Val Glu His Glu Glu Val Asn
    210                 215                 220

Phe Ile Asn Phe Thr Gly Ser Thr Pro Val Gly Gln Arg Ile Gly Lys
225                 230                 235                 240
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Leu Ala Gly Met Arg Pro Ile Met Leu Glu Leu Gly Gly Lys Asp Ala
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Gly Ile Val Leu Ala Asp Ala Asp Leu Asp Asn Ala Ala Lys Gln Ile
            260                 265                 270

Val Ala Gly Ala Tyr Asp Tyr Ser Gly Gln Arg Cys Thr Ala Ile Lys
        275                 280                 285

Arg Val Leu Val Val Glu Glu Val Xaa Asp Glu Leu Ala Glu Lys Ile
    290                 295                 300

Ser Glu Asn Val Ala Lys Leu Ser Val Gly Asp Pro Phe Asp Asn Ala
305                 310                 315                 320

Thr Val Thr Pro Val Ile Asp Asp Asn Ser Ala Asp Phe Ile Glu Ser
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Leu Val Val Asp Ala Arg Gln Lys Gly Ala Lys Glu Leu Asn Glu Phe
            340                 345                 350

Lys Arg Asp Gly Arg Leu Leu Thr Pro Gly Leu Phe Asp His Val Thr
        355                 360                 365

Leu Asp Met Lys Leu Ala Trp Glu Glu Pro Phe Gly Pro Ile Leu Pro
    370                 375                 380

Ile Ile Arg Val Lys Asp Ala Glu Glu Ala Val Ala Ile Ala Asn Lys
385                 390                 395                 400

Ser Asp Phe Gly Leu Gln Ser Ser Val Phe Thr Arg Asp Phe Gln Lys
                405                 410                 415

Ala Phe Asp Ile Ala Asn Lys Leu Glu Val Gly Thr Val His Ile Asn
            420                 425                 430

Asn Lys Thr Gly Arg Gly Xaa Asp Asn Phe Pro Phe Leu Gly Leu Lys
        435                 440                 445

Gly Ser Gly Ala Gly Val Gln Gly Ile Arg Tyr Ser Ile Glu Ala Met
    450                 455                 460

Thr Asn Val Lys Ser Ile Val Leu Asp Met Lys
465                 470                 475
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